

STUDY PROTOCOL

PROTOCOL TITLE:

Effects of local minocycline adjunctive application in comparison to instrumentation alone, in patients with residual pockets under supportive periodontal therapy: a double-blinded randomized controlled clinical trial.

PROTOCOL NUMBER:

196/2014

PROTOCOL VERSION: 7**PROTOCOL DATE:** 15.04.2016**PRINCIPAL INVESTIGATOR:**

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PROTOCOL SIGNATURE PAGE

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Sponsor Name: National Dental Centre Singapore

Funding Source: Sunstar Singapore Pte. Ltd.

Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described trial in compliance with all stipulations of the protocol, regulations and Singapore Guideline for Good Clinical Practice (SGGCP).

Principal Investigator Name: Dr Wah Ching Tan

Principal Investigator Signature:

Date:

Abstract:

The aims of the present study are to assess the significance of the adjunctive effect of the subgingival application of a 2% minocycline hydrochloride controlled-delivery system (MHS) in comparison to subgingival instrumentation with application of a placebo gel, 3 months after therapy in subjects with recurrent periodontitis undergoing supportive periodontal therapy (SPT) and to assess the substantivity of the gel attributable to the adjunctive delivery of the medication that is detected at 3 months during a 9-month period of regular SPT. Recurrent periodontitis will be defined as sites with residual periodontal probing depths and bleeding on probing after completion of initial periodontal therapy. This will include both persistent and recurrent periodontitis, where persistent means the residual periodontal site after initial periodontal therapy, and recurrent means the site which was improved by initial periodontal therapy, but disease recurred. This will be a randomised, double-blinded trial in parallel groups.

Patients undergoing regular maintenance care in the National Dental Centre Singapore, Periodontics Unit and suffering from recurrent moderate to severe periodontitis were randomized to two treatment groups:

- (i) Test: Mechanical ultrasonic/ hand instrumentation and subsequent administration of MHS on that day (Day 0) and on Day 4, at 3 months, 6 months and 9 months
- (ii) Control: Mechanical ultrasonic/ hand instrumentation and subsequent administration of a placebo gel on that day (Day 0) and on Day 4, at 3 months, 6 months and 9 months

All patients will be treated with supragingival debridement as required and subgingival ultrasonic/ hand instrumentation (without using antimicrobial agents as irrigating solutions) at all sites with pocket depths of 5mm or deeper.

There will be no time limitations. All the dentition will be polished to complete the SPT appointment. The instrumentation time will be recorded. After completion of the ultrasonic/ hand instrumentation, the assigned randomization envelope will be opened, and test subjects will receive application of the test agent in all pockets 5 mm or deeper, while the placebo subjects will receive application of a placebo gel. The time required for application of the agents will also be recorded along with the number of used doses. Patients (both test and control) will be instructed not to eat and/or drink for the next 2 hours and to avoid any form of interdental cleaning for the first 12 hours following treatment. Normal oral hygiene procedures will resume after 12 hours.

Since there are few studies on the effect of 2% minocycline hydrochloride controlled-delivery system (MHS) in subjects with recurrent periodontitis undergoing supportive periodontal therapy, this will be a valuable study.

1. BACKGROUND AND RATIONALE

1.1. General Introduction

It has been reported that regular maintenance of subjects with treated periodontal disease is the key consideration in the long-term periodontal prognosis of these subjects. Periodic prophylaxis may prevent loss of clinical attachment over long periods of time even in patients with less than optimal plaque control (Ramfjord 1987). However, there are limitations in routine subgingival re-instrumentation especially in bleeding pockets, as only 50% of these sites improve (Tonetti et al. 1998). Furthermore, the persistence of bleeding pockets increases the risk of disease progression and tooth loss (Matuliene et al. 2008, 2010). Thus there is a need for adjuncts that may improve the outcome especially in subjects with recurrent periodontitis during SPT. Some studies reported significantly better results with subgingival administration of local minocycline in residual pockets post initial periodontal therapy over a short period of time (Lu et al 2005). There are few studies assessing the efficacy of topical minocycline gel in subjects with recurrent periodontitis while in SPT, and the long term effect.

The present study will examine the significance of the adjunctive effect of the subgingival application of a 2% minocycline hydrochloride controlled-delivery system (MHS) in comparison to subgingival instrumentation with application of a placebo gel, 3 months after therapy in subjects with recurrent periodontitis undergoing supportive periodontal therapy (SPT) and to assess the substantivity of the gel attributable to the adjunctive delivery of the medication that will be detected at 3 months during a 9-month period of regular SPT.

1.2. Rationale and justification for the Study

a. Rationale for the Study Purpose

There are currently few studies that examine the effect of a 2% minocycline hydrochloride controlled-delivery system (MHS) in subjects undergoing supportive periodontal therapy, with residual PPD and bleeding on probing.

b. Rationale for Doses Selected

Minocycline hydrochloride concentrations in the pockets decreased rapidly for the first 7 hours after administration. Subsequent decrease was very slow, with the concentration at 72 hours being 3.4µg/ml. At 100 hours, the concentration of minocycline is still effective against *Prophyromonas gingivalis*, *Prevotella intermedia*, and *Eikenella corrodens*.

At 168 hours, the concentration reduced to 0.1µg/ml making it ineffective against *Prophyromonas gingivalis*, *Prevotella intermedia*, and *Eikenella corrodens*. (Satomi et al. 1987) Thus in this study, re-administration of the gel will be at Day 4, before the concentration is reduced to 0.1µg/ml.

c. Rationale for Study Population

The study population will be patients undergoing regular maintenance care in the National Dental Centre Singapore, Periodontics Unit, Department of Restorative Dentistry and having recurrent moderate to severe periodontitis (residual probing depths ≥ 5 mm and bleeding in probing). Regular maintenance care is defined as professional periodontal recall of patients at a tailored time frame for examination of periodontal status and administration of treatment, including scaling and polishing, root planning of sites with probing depths of 5mm and above, and oral hygiene reinforcement.

d. Rationale for Study Design

The study will be a randomised, double-blinded controlled clinical trial.

2. HYPOTHESIS AND OBJECTIVES

2.1. Hypothesis

The null hypothesis of this trial is that there is no difference in clinical outcomes with the adjunctive administration of a 2% minocycline controlled-delivery system (MHS) (test) together with subgingival instrumentation when compared to subgingival instrumentation with placebo gel (control), in subjects with recurrent and/or persistent probing depths, receiving supportive periodontal therapy (SPT).

2.2. Primary Objectives

To determine at 3, 6, 9 and 12 months after intervention, based on average of all sites ≥ 5 mm per patients with BOP at baseline:

- Absolute change of probing pocket depth (PPD): change of PPD at each time point and baseline, calculated as primarily patient-based analysis, and site based as well

- Percentage reduction of number of PPD ≥ 5 mm: total number of sites with PPD ≥ 5 mm at baseline- number of sites with PPD ≥ 5 mm at each time point/total number of sites with PPD ≥ 5 mm at baseline, calculated both as patient-based and site based.

2.3. Secondary Objectives

- Reduction in bleeding on probing (BOP): this includes full mouth bleeding score and bleeding score of the treatment sites alone

- Change of probing attachment level (PAL): similar to PPD calculation

- Incidence of recurrent rate during experimental period

2.4. Study End points

Primary endpoint:

Absolute change of PPD at 3 months compared to baseline is defined as the difference between absolute patient PPD at 3 months and absolute patient PPD at baseline. For patients who have multiple trial sites, the mean of site absolute PPD will be used as their patient PPD score.

Secondary endpoints:

Absolute change of PPD at 6, 9, or 12 months compared to baseline are defined as the difference between absolute patient PPD at 6, 9, or 12 months and absolute patient PPD at baseline. For patients who have multiple trial sites, the mean of site absolute PPD will be used as their patient PPD score.

Percentage reduction of number of PPD ≥ 5 mm at 3, 6, 9, 12 months are defined as the following formula:

percentage reduction of number of PPD ≥ 5 mm at 3,6,9,12 months= (no.of sites with PPD ≥ 5 mm at baseline-no.of sites with PPD ≥ 5 mm at 3,6,9,12 months)/(no.of sites with PPD ≥ 5 mm at baseline)

Reduction in bleeding on probing (BOP) is defined as the difference between full mouth BOP score for each patient at two different study visits as well as the difference between bleeding score of the treatment sites at two different study visits. The comparison will be made between the following study visits: 3 months vs baseline, 6 months vs baseline, 9 months vs baseline, 12 months vs baseline, 3 months vs 6 months, 3 months vs 9 months, and 3 months vs 12 months.

Change of probing attachment level (PAL) at 3, 6, 9, 12 months compared to baseline are defined as the difference between patient PAL score at 3, 6, 9, 12 months and patient PAL score at baseline. For patients who have multiple trial sites, the mean of site PAL will be used as their patient PAL score.

Incidence of recurrent rate during experimental period. The recurrent means the case of periodontal progression (attachment loss of 2mm or more) between test and groups.

2.5. Potential Risks and benefits:

a. End Points - Efficacy

For the test group, potential benefits may include improvement in the clinical outcomes.

b. End Points - Safety

For the test group, the risk involved would be the development of antibiotic resistance and known side-effects of the drug. As for the control group, there may not be any improvement in the clinical outcomes.

2.6. List the number of subjects to be enrolled.

230 subjects will be selected for inclusion into the study when they present for their regular supportive periodontal therapy appointments. Subjects fulfilling the inclusion criteria will be invited to participate in the study.

2.7. Criteria for Recruitment

All subjects had been previously been diagnosed with periodontitis and had received a full cycle of periodontal therapy consisting of oral hygiene instructions, scaling and root-planing. All subjects had received a minimum of 6 months of documented supportive periodontal care.

2.8. Inclusion Criteria

A subject must meet all of the inclusion criteria listed below to participate in this study:

- 1) Medically healthy adults (ASA classification I-II), at least 21 years of age.
- 2) Previously diagnosed with moderate to severe periodontitis and had completed at least 1 round of periodontal therapy including scaling and root-planing, oral hygiene instructions.
- 3) Treated periodontitis patients undergoing maintenance care for at least 6 months.
- 4) Ability to comply with 12-month study follow-up.
- 5) Recurrent moderate to severe periodontitis with no previous systemic antibiotic therapy during initial periodontal therapy.
- 6) At least 4 teeth present with residual PPD of ≥ 5 mm on each and a positive bleeding on probing (BOP).

2.9. Exclusion Criteria

Subjects with any of the following exclusion criteria at baseline, will be excluded from the study:

- 1) Medically compromised subjects (ASA classification III-V).
- 2) Known allergy or other severe adverse reactions to minocycline and related drugs.
- 3) Patients who reported local and/or systemic antibiotic therapy within 3 months prior to baseline examination of the study, and were placed on antibiotics during active initial periodontal therapy.
- 4) Patients with a plaque control record $> 30\%$.
- 5) Patients who had history of surgical periodontal treatment less than 5 years

in the area with lesions.

- 6) Pregnant or intend to conceive or are breast feeding.

2.10. Withdrawal Criteria

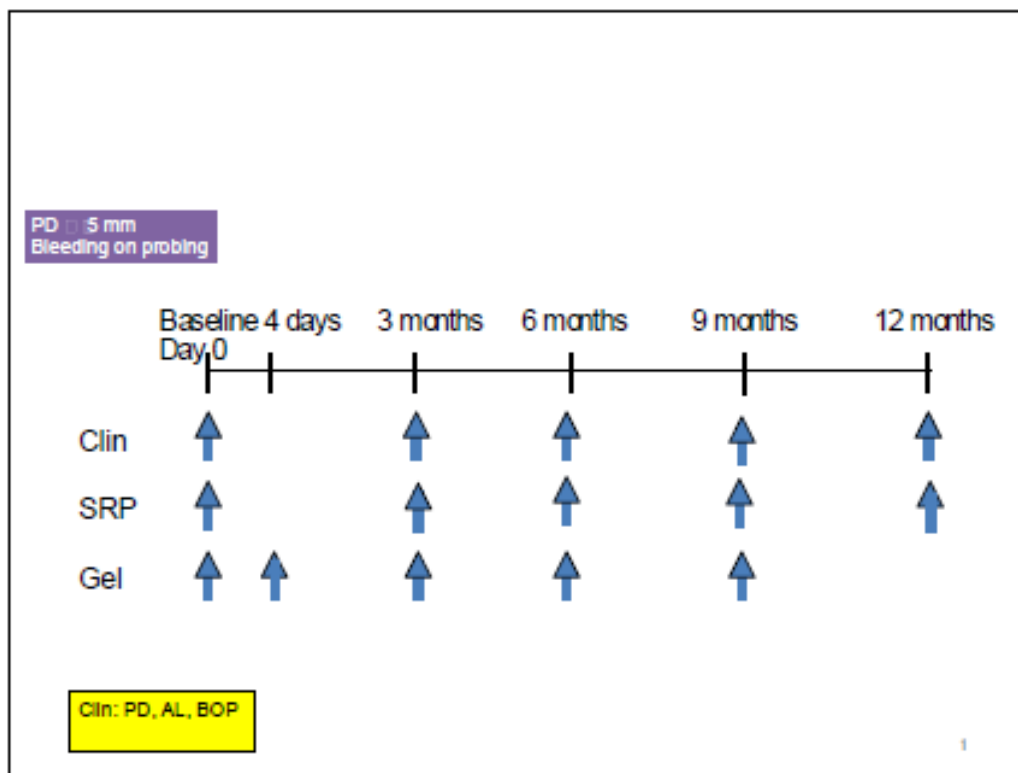
Possible reasons for discontinuation of study intervention may include:

- allergy or adverse reaction to minocycline requiring the medication to be discontinued.
- other forms of antibiotics being taken during the study period.
- subject chooses to exit study at any period of time.
- case of periodontitis progression at any site (attachment loss of 2 mm or more), the patient will be excluded from the study and receive treatment as necessary.

2.11. Subject Replacement

Subjects who drop out will not be replaced. The data collected will still be adopted for analysis to compare the cases who had periodontitis progression between the test and control groups.

3. TRIAL SCHEDULE



Sequence of events

Clin: PD, AL, BOP measured.

SRP: ultrasonic/hand instrumentation at sites with PPD ≥ 5 mm and positive bleeding on probing.

Gel: either test or placebo gel applied

4. STUDY DESIGN

4.1. Summary of Study Design

This will be a randomised double-blinded controlled clinical trial in parallel groups, with the aim to assess the adjunctive effect of a 2% minocycline hydrochloride controlled-delivery system (MHS) compared to mechanical maintenance therapy 3, 6, 9 and 12 months after intervention.

5. METHODS AND ASSESSMENTS

Sunstar Singapore will act as a project monitor.

5.1. Definitions

a. Examiner

The examiners are specialist periodontists who will perform clinical examinations at baseline and at the recall (3, 6, 9 and 12 months). This will be performed by 3 calibrated examiners (Drs Marianne Ong, Wah Ching Tan and Koh Chu Guan).

b. Clinicians

Clinicians will be those who will be involved in instrumentation of the tooth sites with probing pocket depths of 5 mm or more, and in the application of the topical gel.

c. Calibration

The 3 examiners will undergo intra- and inter-examiner calibration as described below:

6 subjects with presence of Ramfjord teeth (#16, 11, 24, 36, 31, 44) as being representative of the dentition and teeth contralateral to the Ramfjord teeth (#14, 21, 26, 34, 41, 46) will be selected for the calibration exercise. These 6 subjects will be scored four times each, not consecutively, but in a random fashion. The first and third scorings will use the teeth proposed by Ramfjord as being representative of the dentition, while the second and fourth examinations will utilise the contralateral Ramfjord teeth as being representative of the dentition. The probing depths of the 6 Ramfjord teeth will be scored on 4 surfaces (three buccal: mesio-buccal, buccal and disto buccal and one lingual aspect) and a mean score will be calculated. This same process is performed on the contralateral teeth. The second examiner will go through the same process. Each subject will be scored twice by each examiner. Examiner agreement will be measured through the intraclass correlation coefficient. Calibration will be for PPD and PAL.

d. Method of Assessment

Probing pocket depth and marginal recession will be measured to the nearest millimetre with a UNC-15 probe (Hu-friedy®) with 1 mm incremental markings. Probing attachment loss is calculated as the sum of the distance from the gingival margin to the CEJ (cement-enamel junction) and probing pocket depth. Bleeding on probing will be assessed dichotomously with a UNC-15 probe (Hu-friedy®). Full mouth bleeding scores (BOP) and plaque score will be calculated. BOP is the presence or absence of bleeding on probing, recorded following a full mouth charting.

e. Treatment Sites

Treatment sites will be tooth sites with recurrent or persistent probing depths of 5

mm or greater and positive to bleeding on probing.

f. Randomisation and Blinding

A randomisation table will be used to randomly assign the subjects to either the test or control group. Blocked randomisation will be performed in blocks of ten whereby at every block of 10 enrolments, there will be 5 subjects randomly assigned either to the test or control groups. The randomisation table will be stratified to ensure smokers will be evenly distributed between the test and control groups. This will yield 2 strata. Within each stratum, the randomization sequence will be generated by the SAS Software 9.1, with a 1:1 allocation ratio using random block sizes of size 10 by a statistician to ensure that at every block of 10 enrolments, there will be 5 subjects randomly assigned either to the test or control groups. The examiners will be blinded during the clinical examination of the patients. The topical antibiotic will be administered by a designated clinician, who is not involved as the examiner.

Randomisation will be performed at the time of consent.

5.2. Contraception and Pregnancy Testing

N/A.

5.3. Study Visits and Procedures

Refer to flow chart in Section 4.

a. Screening Procedures

Patients are screened at their routine supportive therapy visit. Patients who fulfil the inclusion criteria will be invited to participate in the study. Informed consent will be taken at the baseline visit.

b. Study Visits and Procedures

At baseline (Day 0), full mouth bleeding and plaque score will be recorded. All patients will be treated with supragingival debridement as required and subgingival ultrasonic/hand instrumentation (without using antimicrobial agents as irrigating solutions) at all sites with pocket depths of 5 mm or deeper. There will be no time limitations. All the dentition will be polished to complete the SPT appointment. The instrumentation time will be recorded. After completion of the ultrasonic/hand instrumentation, the assigned randomization envelope will be opened by the research co-ordinator and test subjects will receive application of the test agent in all pockets 5 mm or deeper, and in the adjacent teeth close to the test sites, while the control subjects will receive a placebo gel. The time required for application of the test agent will also be recorded along with the number of used doses. Patients (both test and control) will be instructed not to eat and/or drink for the next 2 hours and to avoid any form of interdental cleaning for the first 12 hours following treatment. Normal oral hygiene procedures will resume

after 12 hours. At day 4, another application of gel will be administered for each respective group.

At 3, 6, 9 and 12 months after treatment, patients will be recalled. At 3, 6, and 9 months, clinical re-examinations as well as mechanical instrumentation and gel application will be administered for each respective group. SPT follow-up will be performed every three months for a period of 1 year.

c. Final Study Visit:

1 year from the first application of a 2% minocycline hydrochloride controlled-delivery system (MHS).

d. Post Study Follow up and Procedures

N/A

e. Discontinuation Visit and Procedures

N/A

6. TRIAL MATERIALS

6.1. Trial Product (s)

The test product is a highly viscous gel for local subgingival placement composing of an ointment containing micro-capsule type particles for sustained release and the active ingredient 2% minocycline gel (10mg in each syringe of 0.5g) (Perioclone, SUNSTAR, Osaka, Japan). The other ingredients include magnesium chloride, hydroxyl-ethylcellulose, aminoalkylmethacrylate copolymer, triacetin and concentrated glycerine, giving the preparation a sustained-released property. It will be applied to experimental teeth and the adjacent teeth by gently inserting the tip of a specially designed applicator until the paste flows over the gingival margin.

6.2. Storage and Drug Accountability

The product will be stored at 2-8 degrees Celsius.

7. TREATMENT

7.1. Rationale for Selection of Dose

Minocycline hydrochloride concentrations in the pockets decreased rapidly for the first 7 hours after administration. Subsequent decrease was very slow, with the

concentration at 72 hours being 3.4µg/ml. At 100 hours, the concentration of minocycline is still effective against *Prophyromonas gingivalis*, *Prevotella intermedia*, and *Eikenella corrodens*. At 168 hours, the concentration reduced to 0.1µg/ml (Satomi et al. 1987). Thus, in this study, a second application will be timed for Day 4 before the concentration is reduced to 0.1µg/ml. (Figure 1)

7.2. Study Drug Formulations

The test product is a highly viscous gel for local subgingival placement composing of a high viscous gel containing micro-capsule type particles for sustained release and the active ingredient 2% minocycline hydrochloride (10mg in each syringe of 0.5g) (Perioline, SUNSTAR, Osaka, Japan). The other ingredients include magnesium chloride, hydroxyl-ethylcellulose, aminoalkylmethacrylate copolymer, triacetin and concentrated glycerine as inactive ingredients, giving the preparation a sustained-released property. The placebo gel is the same gel as test but without active ingredient minocycline hydrochloride.

7.3. Packaging and Labeling

Every syringe for test is packaged in a heat-sealed foil laminated pouch which is light-proof and printed 'PERIOCLINE'. Every syringe for placebo is packaged in the same pouch as test product but attached with a label printed 'Placebo' on the pouch.

A label printed with 'For Clinical Trial Use Only Effects of local minocycline adjunctive application in comparison to instrumentation alone, in patients with residual pockets under supportive periodontal therapy: a blinded randomized controlled clinical trial To store at 2-8°C' is attached on every pouch (test and control).

7.4. Study Drug Administration

It will be applied to the teeth with experimental sites and the adjacent teeth by gently inserting the tip of a specially designed applicator until the paste flows over the gingival margin.

Test and control syringes are disposable and can be used only in one patient per visit. Every syringe (test and control) can be used to treat multiple pockets in the same patient but not multiple patients.

To avoid the administration pain caused by the cold formulation, every pouch containing syringe (test and control) should be left in room temperature for 10 minutes before administration.

7.5. Specific Restrictions / Requirements

The patient will be instructed not to eat and/or drink for 2 hours after drug

administration, and to avoid brushing, rinsing and flossing for 12 hours after treatment.

7.6. Blinding

The examiners will be blinded to the type of gel received by patients – placebo vs antibiotic.

7.7. Concomitant therapy

All medications (prescription and over the counter), vitamin and mineral supplements, and / or herbs taken by the participant will be documented.

8. SAFETY MEASUREMENTS

8.1. Definitions

Periodontitis progression at any site (defined as clinical attachment loss of 2 mm or more).

8.2. Collecting, Recording and Reporting of Adverse Events

Adverse events will be recorded & reported on by the project monitor.

8.3. Safety Monitoring Plan

Patients will be issued a study number & de-identified on the data collection forms which will be stored under lock& key by the PI. The data will be entered into an Excel spreadsheet and stored in a password-protected laptop accessible only to the PI.

8.4. Complaint Handling –

Complaints will be handled by the principal investigator and co-investigators, and the data collected from the study subjects will be used with their consent only.

9. ADVERSE EVENTS

The occurrence of adverse effects (AE) will be monitored throughout all phase of the study and will be assessed in all participating subjects. Adverse events are to be elicited by examiner by asking the patient non-leading questions. The association of the AE to Perioline is to be judged by the investigator. All adverse events will be recorded on the individual Case Report Form by the Investigators.

9.1. Definitions

a. An Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign, symptom or disease temporarily associated with the use of this medicinal (investigational) product, whether or not related to this medicinal (investigational) product.

b. A Serious Adverse Event (SAE)

Any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/ incapacity, or results in a congenital anomaly/ birth defect.

NOTE: Important medical events which may not result in death, be life threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

c. Unexpected Adverse Drug Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or package insert/ summary of product characteristics for an approved product.)

9.2. Assessment of Severity (Intensity)

All adverse events will be examined by the Investigator for assessment of both severity and causality using the following criteria:

a. Mild

Awareness of a sign or symptom, but subject can tolerate

b. Moderate

Discomfort is sufficient to cause interference with normal daily activity.

c. Severe

Resulting in an ability to do work or do usual daily activity.

9.3. Reporting of SAEs

A written SAE report by the Investigator to the Sponsor will follow immediately

of the Investigator's knowledge of the occurrence of the SAE. The Investigators must also report all serious adverse reactions to cIRB and HSA within the timeline stated by the respective organizations.

10. DATA ANALYSIS

Data Collection Forms attached
Form A & Form B: Data collection

10.1. Data Quality Assurance

Data will be recorded by the Principal Investigator and Co-Investigators.

10.2. Data Entry and Storage

Data will be first entered on paper. Upon completion of data collection, the information will be transferred to an Excel spreadsheet prior to data analysis.

10.3. Handling of Missing Data, Withdrawals and Treatment Failure

Research data from withdrawn subjects and/or treatment-failed subjects will be retained for the purpose of data analysis. Research data that has been collected will be retained for a minimum of 6 years after the completion of the clinical trial.

11. SAMPLE SIZE AND STATISTICAL METHODS

11.1. Determination of Sample Size

It is assumed that the difference in absolute change in PPD at 3 months compared to baseline between test and control group is 0.5mm and a standard deviation of 1.2mm (Dannewitz et al. 2009). With a 1:1 allocation ratio, to attain 80% power and two-sided 5% type I error, the required sample size is 184 patients (92 per group) by a two-sample t-test. If a drop-out rate is 20% is allowed, the required number increases to approximately 230 patients (115 per group).

11.2. Statistical and Analytical Plans

a. Definition of Analysis sets

- Intent-to-Treat (ITT) analysis set: All patients who are randomized will be included in the ITT analysis set. Treatment group will be according to the pre-planned randomization list.
- Treated analysis set: All patients who are randomized and receive treatment will be included in treated analysis set. Treatment group will be according to the actual treatment that patients receive. For example, if a patient is randomized to test but receives control instead, control will be the treatment group for this

patient under treated analysis set.

- Per protocol (PP) analysis set: all ITT patients without any major protocol deviations

b. General Comment

Baseline characteristics will be reported using descriptive statistics only. Mean, standard deviation, and interquartile range will be reported for continuous variables, and number of observations and proportion will be reported for categorical variables.

An independent sample t-test (two-tailed) will be used to compare the difference in the following study outcomes between test and control groups:

- the Absolute change of PPD at 3, 6, 9, 12 months compared to baseline
- Percentage reduction of number of PPD ≥ 5 mm at 3, 6, 9, 12 months compared to baseline
- Reduction in bleeding on probing (BOP) at different study visits compared to baseline
- Change of probing attachment level (PAL) at 3, 6, 9, 12 months compared to baseline

Difference in mean, 95% confidence interval and p-value will be reported for each analysis. 5% will be set as significance level. Non-paramateric test will used when appropriate if any of the study outcomes is not normally distributed.

Regression analysis on absolute change of PPD at 3 months compared to baseline will be performed to adjust for several other risk factors, including smoking, plaque score (at baseline), and bleeding on probing (at baseline). If there is any baseline imbalance of certain patient characteristics between two groups, such variable will also be adjusted in regression analysis. Coefficient from regression analysis for each factor in the model, 95% confidence interval for each coefficient, and adjusted p-value will be reported.

Descriptive statistics will be used to determine if the absolute change of patient PPD at 3 months is sustained over time of 1 year. For patients with multiple trial sites, the mean of absolute change of site PPD will be used as absolute change of patient PPD.

c. Safety Analyses

Number and percentage of patients experiencing 1 or more AEs/SAE will be summarized by expectness, relationship to study drug, outcome and severity. Number of patients experiencing at least 1 AE/SAE will be tabulated and compared between two groups by exact method. Odds ratio, and its 95% confidence interval will be reported

d. Interim Analyses:

An independent Data Safety Monitoring Board (DSMB) will be formed to review

the safety in this study. The DSMB will be comprised of three members: two clinician, and a biostatistician, all independent from the trial. One interim analysis of safety will be performed by the DSMB after 50% of the subjects are 3 months into the study. The analysis report will be produced using dummy treatment group. Only primary efficacy endpoint without p-values and safety endpoints will be presented to DSMB members. Decision to terminate the trial prematurely will depend not just on the interim results but also on other relevant information such as the patients' prognosis and the nature of treatment. DSMB members will inform the study principle investigator if any safety concern is shown from the interim analysis. The data for such a review will remain confidential to the designated trial biostatistician and the DSMB members. The Board will report their recommendations to the study principle investigators for them to take any necessary action(s).

12. ETHICAL CONSIDERATIONS

12.1. Informed Consent

Each subject will be provided with a research folder where the research data and informed consent will be filed. Interested subjects will be provided with time to read through the consent form, with verbal explanation on the procedures and the risks involved. This will be explained by the examiner, and any queries or concerns will be answered. A witness must be present at the point of consent taking. Consent will be taken at the time of recruitment into the study by the examiner. The consent used will be for study participation. The study folders will be stored under lock and key.

12.2. IRB review

The study protocol and the associated informed consent documents will be reviewed by the cIRB.

12.3. Confidentiality of Data and Patient Records

No other person/ persons other than the investigators have access to the information gathered through the study. Each patient will be given a study code; the master list links patient's identity and the study code will be kept separately from the study data and will be in the possession of the Principal Investigator. Data Collections Forms will only bear the patient's allocated study code as the only form of identification.

All hard copies of data collected will be kept under lock and key in the cabinets in the research office. Soft copies of the data will be stored in a password-protected laptop accessible only to the Principal Investigator.

13. PUBLICATIONS

The study findings will be published in a peer-reviewed journal.

14. RETENTION OF TRIAL DOCUMENTS

Records for all participants, including all source documentations as well as cIRB records and other regulatory documentations, will be retained by the Principal Investigator in a secure storage facility. The records will be accessible for inspection and copying by authorized authorities.

15. COSTING

A budget sheet shows the costs and other sources of funding for the project should be submitted.

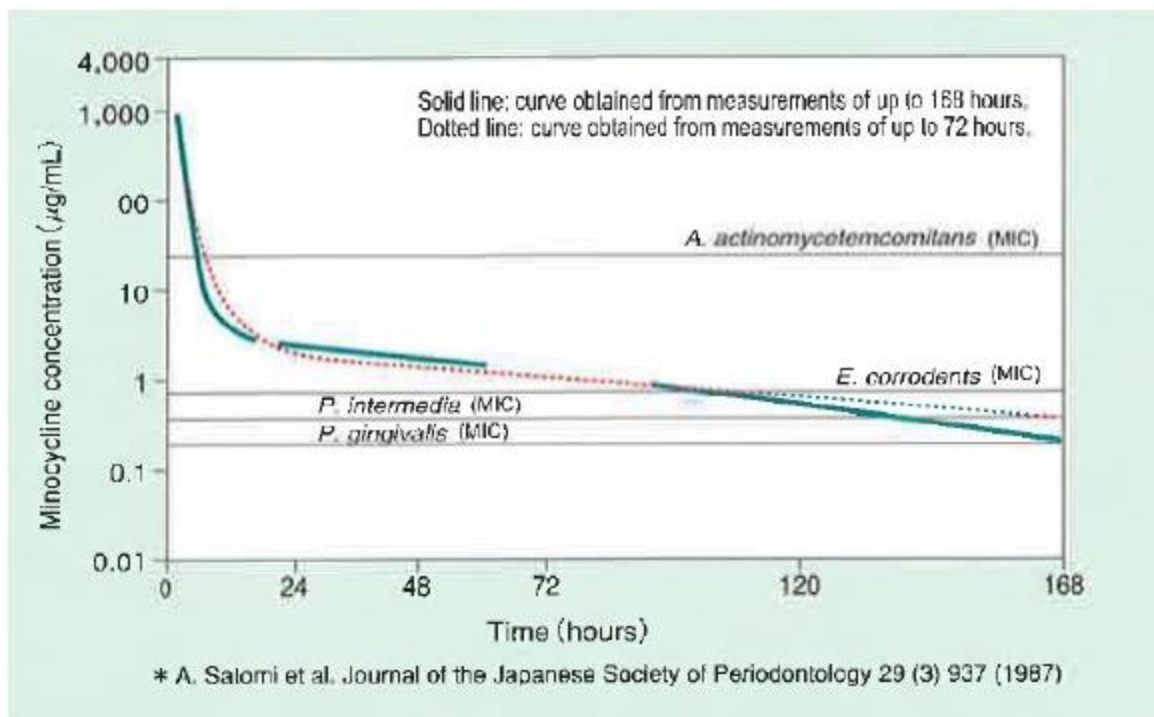


Figure 1. Topical minocycline concentration with time

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